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Development of carpels and ovules in *Psychotria carthagenensis* (Psychotrieae) and *Rudgea macrophylla* (Palicoureeae) (Rubioideae, Rubiaceae)

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ABSTRACT

The study of floral ontogeny across the entire Rubiaceae family is essential to an understanding of its floral evolution. However, studies reporting on the development of the gynoecium, as well as the formation of the carpel septa, placenta and ovules are scarce. This work, therefore aimed to assess placentation and gynosporogenesis, as well as carpel, septum, and ovule development, in *Psychotria carthagenensis* Jacq. (Psychotrieae) and *Rudgea macrophylla* Benth. (Palicoureeae). A new ovule type, *Psychotria*, is here recognized to accommodate the unique combination of ovule characters observed in these two species and possibly the Psychotrieae–Palicoureeae clade. In this ovule type the carpels are considered as sterile appendices, and the placenta represents a continuation of the floral meristem based on its position relative to the carpels. The ovules are erect and located at the basal portion of the locule. The nucellar epidermis appears at early developmental stages as a flat surface and, later on, as a dome-like structure. The new type is closely related to the *Phyllis* type but is distinguished by the erect ovules as opposed to pendulous in the *Phyllis* type.

1. Introduction

The Rubiaceae family comprises approximately 13,548 species and 617 genera (The Plant List, 2010). Despite its broad geographic distribution, it is more frequently found in the tropics (Robbrecht, 1988). Robbrecht and Manen (2006) recognize two subfamilies: Cinchonoideae and Rubioideae. Within Rubioideae, ca. 18 tribes are recognized, including Palicoureeae and Psychotrieae *s.str*. The sister tribes Palicoureeae and Psychotrieae have been demonstrated to be monophyletic and together form a monophyletic group, hereafter referred to as the Psychotrieae–Palicoureeae clade (Robbrecht and Manen, 2006; Razafimandimbison et al., 2008).

Psychotria carthagenensis Jacq. and *Rudgea macrophylla* Benth. both belong to the Psychotrieae–Palicoureeae clade. The former is included within the tribe Psychotrieae, in the Neotropical *Psychotria* clade (Andersson, 2002), while the latter is included in the tribe Palicoureeae (Razafimandimbison et al., 2008). *Psychotria* L. comprises about 1900 pantropical species (The Plant List, 2010) and is especially abundant and diverse in the Brazilian Atlantic Forest (Martini et al., 2007), which harbors approximately 104 species of this genus (Taylor, 2012). *Rudgea* Salisb. comprises approximately 120 Neotropical species, distributed from Mexico to Argentina, and has its centers of diversity in the northwestern South America and the Atlantic Forest of southeastern Brazil (Zappi, 2003). Previous embryological studies on the development of carpels and ovules focused on taxa distributed mostly in temperate regions and belonging to the tribes Rubieae and Spermacoceae of subfamily Rubioideae. Until now there are no detailed data about the development of carpels and ovules in the Psychotrieae–Palicoureeae clade.

Phylogenetic hypotheses based on molecular data may help interpret morphological and anatomical data (Robbrecht and Manen, 2006). Accordingly, embryological studies have provided morphological data to test molecular phylogenies, such as those of Tokuoka and Tobe (1995, 2002) in Euphorbiaceae, as well as Heo et al. (1998) and Kimoto et al. (2006) in Lauraceae. Specifically, the structure of the ovule has been used in systematic and phylogenetic discussions. Bouman (1984), for instance, recognizes two types of ovule initiation: bi- or trizonate ovular primordium, indicating that ovule development begins with periclinal divisions of the second or third layer of the placental dome. Among angiosperms, the trizonate ovular primordium is the most common (Bouman, 1984) and may be considered a plesiomorphic character. Variations in the nucellus are also considered. Shamrov (1998) recognized two varieties in tenuinucellate ovules of flowering plants: the typical, or sympetalous, and the reduced types. For Rubiaceae, Fagerlind (1937) recognized three types: sympetalous, reduced and naked. Both classifications considered variations in nucellar shape (dome-like or flat).

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The objective of this paper is to provide detailed descriptions of the development of carpels and ovules, including the gynosporogenesis, with a special emphasis on the placenta, nucellus, and integument in *P. carthagenensis* and *R. macrophylla* and to ascertain whether this embryological data can provide characters which may, upon further study, prove to be of systematic value (i.e. possible synapomorphies for the two tribes, Psychotrieae and Palicoureeae, and/or the Psychotrieae–Palicoureeae clade).

2. Materials and methods

Floral buds and flowers of *P. carthagenensis* Jacq. and *R. macrophylla* Benth. at different developmental stages were collected in the Arboretum of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Brazil. Vouchers were deposited in RB under the collection numbers RB 462316 and RB 276503, respectively.

Samples were fixed in glutaraldehyde 2.5% in sodium phosphate buffer 0.1 M, pH 7.2 (Gabriel, 1982), dehydrated in an ethanol series, and embedded in hydroxyethylmethacrylate (Gerrits and Smid, 1983). Sections of 2–3 μm were produced using a Shandom Hypercut rotary microtome equipped with a Leica D profile steel blade, followed by staining with toluidine blue 0.05% (O'Brien et al., 1965). Photomicrographs were prepared using a CoolSnap Pro digital camera coupled to an Olympus BX-50 optical microscope. The same equipment was used for fluorescence microscopy, but the sections were stained with Aniline Blue 0.5% (Oparka and Read, 1994).

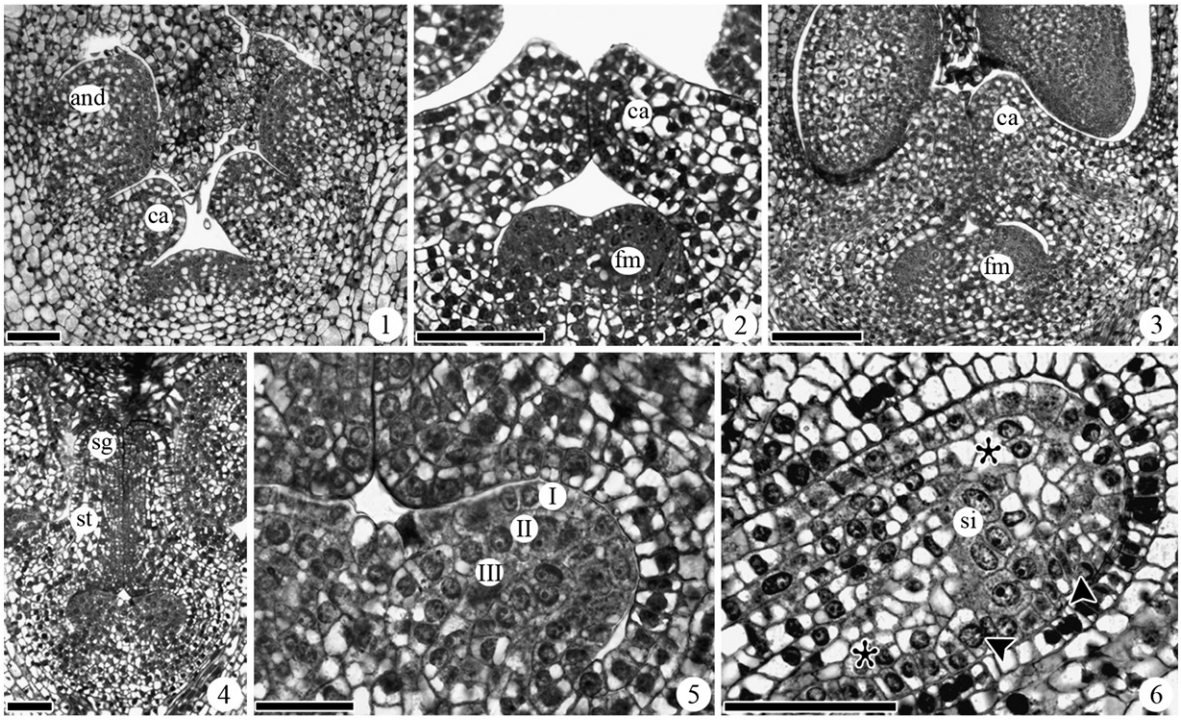
3. Results

No morphological differences in the development of the carpels and ovules of *P. carthagenensis* and *R. macrophylla* were observed. In both species, floral buds, in the early development stages, have the carpels starting to develop after the appearance of the calyx, corolla, and stamens (Fig. 1). Discrete projections of the carpel walls occur initially, followed by their juxtaposition above the early locule (Fig. 2). At this stage, a continuation of the floral meristem at the basal–central portion of the floral bud is observed. After the juxtaposition of the carpel walls, the cells that originally closed the carpel structure keep on proliferating in two directions: (i) towards the corolla, resulting in the formation of the style and stigma, and (ii) towards the base of the carpels, forming the carpelar septum (Figs. 3–4). Then, as the septum reaches the floral meristem (located at the base of the carpels), it delimitates the two locules.

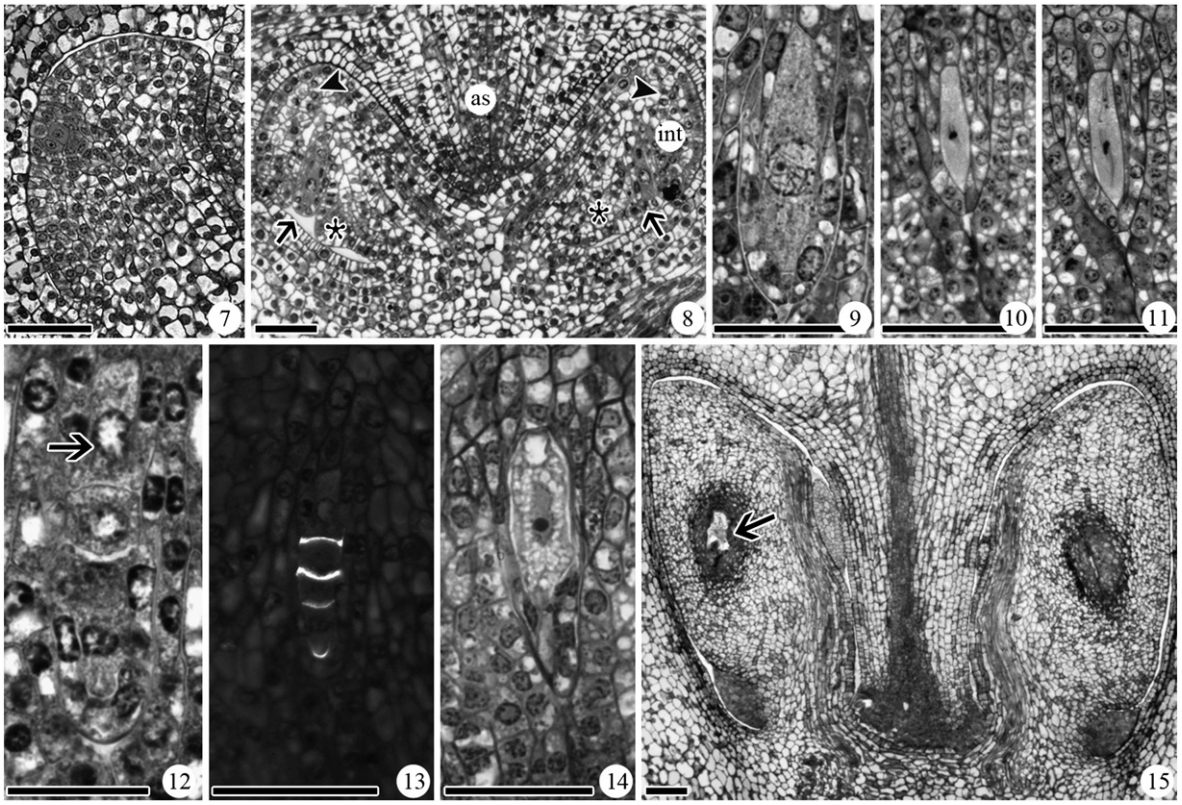
The ovule primordium initiates directly from the continuation of the floral meristem and maintains its location at the basal portion of the locular region (Figs. 2–4). Because the ovule arises directly from the floral meristem, its trilayered meristematic structure is retained: epidermal, subepidermal, and central (Fig. 5). Ovule development initiates with anticlinal, periclinal and oblique cell divisions of the central layer, whereas the epidermal and subepidermal layers grow only through anticlinal divisions.

In the epidermal layer of the apical region of the ovule, a group of approximately five to six cells with dense cytoplasm, conspicuous nuclei, and a few small vacuoles form the nucellar epidermis (Fig. 6). The nucellar epidermis is initially flat, and the nucellus is included in the chalaza at the earliest development stage (Figs. 6–7), acquiring a dome-like shape at later developmental stages (Fig. 8).

Periclinal divisions are observed adjacent to the cells of the nucellar epidermis (Figs. 6–7), forming a single integument. Subsequent anticlinal and periclinal divisions of the epidermal layer result in the formation of a long micropyle. During integument formation at the earliest developmental stages, a tendency towards an anatropous positioning of the ovule is observed (Figs. 6–7). In the subepidermal layer, periclinal divisions characterizing remnant cells from an outer integument are also observed (Figs. 6–8). At this stage, the species are characterized by a bicarpellate gynoecium with a bilocular ovary and a single erect ovule per locule. The ovules are unitegmic and tenuinucellate, with a conspicuous funicular obturator (Figs. 8, 15).



Figs. 1–6. Longitudinal sections illustrating the development of carpels in *Psychotria carthagenensis* (Figs. 2, 4–6) and *Rudgea macrophylla* (Figs. 1, 3). 1. Early developmental stage of the floral bud: androecium (and) and carpels (ca). 2 and 3. Detail of the juxtaposed carpel walls (ca) and floral meristem (fm). 4. Detail of the style (st) and stigma (sg) appearance. 5. Ovule primordium, three meristematic layers: epidermal (I), subepidermal (II) and central (III). 6. Ovule primordia, periclinal divisions of the early integument, as indicated by arrows, nucellar epidermis (cells between the arrows), subepidermal initials (si), and vestigial integument, as indicated by asterisks. Scale = 50 μm.



Figs. 7–15. Longitudinal sections illustrating the development of ovules and gynosporogenesis in *Psychotria carthagenensis* (Figs. 8–9, 12–13) and *Rudgea macrophylla* (Figs. 7, 10–11, 14–15). 7. Ovule detail, showing the flat nucellar epidermis. 8. Ovary, evidencing the 'apical septum' (as), ovules, nucellar epidermis (arrow), integument appearance (asterisk), and vestigial outer integument (head arrow). 9–11. Detail of the gynospore mother-cell, in prophase, metaphase I and metaphase II, respectively. 12. Detail of the tetrad, with the functional gynospore indicated by an arrow. 13. Detail of the tetrad, with the callose indicated by fluorescence microscopy. 14. Gynophyte mother-cell. 15. Ovary, evidencing the basal erect ovules; the gynophyte is indicated by an arrow. Scale = 50 μm.

Concomitant with the establishment of the nucellar epidermis and integument, a uniform group of cells, known as subepidermal initials, is observed adjacent to the nucellar epidermis in the subepidermal layer. In a longitudinal section, the subepidermal initials constitute a group of approximately five to six cells (Fig. 6). When mature, they become gynospore mother-cells (Fig. 9) and have dense cytoplasm and evident nuclei. Only one of these gynospore mother-cells will undergo successive meiosis (Figs. 10–11), resulting in a linear tetrad of gynospores (Fig. 12), which have callose impregnated in their cell walls. In the chalazal gynospore, the callose is located in the distal periclinal cell wall (Fig. 13). Of the four gynospores formed, only one is viable, while three subsequently degenerate. The viable gynospore forms vacuoles at its cellular extremities, characterizing the beginning of gametogenesis, with the establishment of the gynophyte mother-cell (Fig. 14). After successive mitotic divisions, this gynophyte mother-cell forms the female gametophyte (*Polygonum* type) of monospore origin (Fig. 15).

4. Discussion

4.1. Psychotria ovule type

The two species analyzed, *P. carthagenensis* and *R. macrophylla*, belong to sister tribes within the Psychotrieae–Palicoureeae clade. The present study demonstrates that these species have identical carpel and ovule development quite different from that reported elsewhere in the family and may indicate a possible synapomorphy for the Psychotrieae–Palicoureeae clade. Although this needs to confirmed by further studies on a broader sampling.

The combination of ovary characters found in *P. carthagenensis* and *R. macrophylla* prevents the assignment of the ovules of both species to one of the currently described ovule types. Therefore a new ovule type, *Psychotria*, is here described. The *Psychotria* type is based on the presence of a nucellus that is considered to be intermediate between the sympetalous and reduced types. This new type resembles the *Phyllis* type by the number of archesporial cells and the dome-like shape of the nucellar epidermis. Fagerlind (1937) defined *Psychotria emetica* L.f. (*Ronabea emetica* (L.f.) A. Rich. — tribe Lasiantheae, *sensu* Robbrecht and Manen, 2006) in the *Phyllis* type, but in their study, they did not consider septa development as a relevant character in the evolutionary discussion. Therefore, this species should also be considered in the *Psychotria* type. The ovule is erect in the *Psychotria* type, rather than pendulous in the *Phyllis* type. The *Psychotria* type shares the presence of a vestigial outer integument with both the *Phyllis* and *Mitracarpus* types (De Toni and Mariath, 2004). The latter type includes species of the tribe Spermacoceae, such as *Borreria verticillata* (De Toni and Mariath, 2004). The *Psychotria* type appears to be closely related to the *Phyllis* and *Mitracarpus* types and should be placed between them (De Toni and Mariath, 2010).

4.2. Carpel septa development

Ronse Decraene and Smets (2000) noted that an analysis of the development of carpel septa could be included in phylogenetic discussions. It is well known that the different degrees of 'basal' and 'apical septum' development in Rubiaceae determine ovule position in the carpels (basal, apical or median) (Robbrecht, 1988) and that the septa of mature carpels are heterogeneous (Ronse Decraene and Smets, 2000). In *P. carthagenensis* and *R. macrophylla*, the septum is

composed of a single apical stylar part, i.e., the 'apical septum'; thus, ovule position is basal. In Rubiaceae, Robbrecht (1988) stated that axile placentation is characteristic of the entire family, but its insertion in the septum varies. The ovules may be attached to the entire septum or only a small part of it. Results of the present study do not corroborate those of Robbrecht (1988). Rather, the findings of Ronse Decraene and Smets (2000) are supported because the connection of the 'placenta' (floral meristem) to the septum is clearly postgenital in *Galopina tomentosa*, which precludes an axial origin (Ronse Decraene and Smets, 2000). Hence, sometimes the placenta does not have a carpel origin, but is, instead, a continuation of the floral meristem. In these cases, the carpels should be considered sterile appendices surrounding the ovules (Sattler and Perlin, 1982; Sattler and Lacroix, 1988). Similar observations were made by Chiurugwi et al. (2007), who stated that the floral meristem develops into a dome-shaped placenta in *Impatiens balsamina* L., clearly contradicting the notion that the placenta has axial origin, according to conventional consensus. Ovules arise on the surface of the 'placental dome', which is located at the central basal portion of the floral bud (Chiurugwi et al., 2007), corroborating Sattler and Perlin (1982) and Sattler and Lacroix (1988). Based on this hypothesis, it seems that the dome-shaped placental region in *P. carthagenensis* and *R. macrophylla* is also a continuation of the floral meristem. Hence, the ovules are a terminal whorl and arise independently from the carpels. This finding is supported by molecular analyses, which also suggest an independent origin of carpels and ovules in the sense that ovules may be considered a fifth whorl (Angenent and Colombo, 1996; Nakagawa et al., 2004; Angenent et al., 2005; Yamaki et al., 2005; Colombo et al., 2008).

4.3. Meristematic layers

The evolution of ovule characteristics has been previously investigated by Bouman (1984) in a study on meristematic layers. According to this author, ovules with three layers, i.e., trizonate, are considered plesiomorphic in relation to bizonate, or two-layered, ovules. In Rubiaceae, both types are recorded, and these characters are also included in the morphological analyses. Bizonate ovular primordia has been reported so far only for members of the Rubioideae tribe Rubieae such as *Relbunium* and *Galium* (Mariath and Cocucci, 1997; De Toni and Mariath, 2010) and *Rubia* (Fagerlind, 1937), whereas tri-zonate ovular primordia have been found within members of the subfamily Cinchonoideae (i.e. *Chomelia obtusa*) and Ixoroideae (i.e. *Ixora coccinea*) (De Toni and Mariath, 2008) as well as *P. carthagenensis* and *R. macrophylla* (Rubioideae, Psychotrieae and Palicoureeae).

4.4. Nucellar epidermis and integument

Endress (2011) questioned whether the unitegmic condition originated directly from the reduction of a single integument (outer or inner). This author suggested a complex evolutionary process, in which both integuments take part, although they can no longer be morphologically distinguished. Several authors considered the bitegmic condition to be plesiomorphic in relation to the unitegmic condition (Bouman and Calis, 1977; Doyle and Donoghue, 1986; Endress, 2011). However, some reports suggest that the unitegmic condition evolved through reduction and loss of the outer integument (Bouman and Calis, 1977) and may have been reached several times during the evolution of angiosperms (Bouman, 1984).

In Rubiaceae, the reduction of the outer integument has already been discussed by Fagerlind (1937), Andronova (1977), and De Toni and Mariath (2004). The ovules are unitegmic (Robbrecht, 1988), but traces of an outer integument are recorded here for *P. carthagenensis* and *R. macrophylla*. In the literature, there are similar records for *Ophiorrhiza*, *Coffea*, *Ixora*, *Mussaenda*, *Macrosphyra*, *Tricalysia*, *Scyphiphora*, *Knoxia*, *Cephalanthus*, *Hoffmannia*, *Chiococca*, *Rondeletia*, *Mitragyna* (as *Stephegyne*) (Andronova, 1977), and *B. verticillata* (De Toni and Mariath, 2004). According to Bouman (1984), in the bitegmic ovules of angiosperms, the outer integument develops frequently from the subepidermal layer, whereas the inner integument develops from the epidermal layer. Ontogenetic studies in Rubiaceae corroborated the hypothesis that the vestigial integument derived from the outer integument by subepidermal origin, whereas the inner integument had an epidermal origin (De Toni and Mariath, 2004). These data suggest that the ovules were bitegmic in Rubiaceae ancestors.

The nucellar epidermis and the integument originate from the epidermal layer of the ovular primordium (Fagerlind, 1937; Galati, 1991; Mariath and Cocucci, 1997; De Toni and Mariath, 2004, 2008, 2010), and variations in the arrangement of the nucellar epidermis are known for Rubiaceae (Fagerlind, 1937). The nucellar epidermis of *P. carthagenensis* and *R. macrophylla* only has a flat surface during the earliest developmental stages. Later, the nucellar epidermis of the mature ovule has a dome-like surface, resembling the sympetalous type. We therefore conclude that neither species can be classified into the types proposed by Fagerlind (1937), as they have characteristics suggesting an intermediate position between the sympetalous and reduced types, i.e., an evolutionary intermediate type.

References

Andersson, L., 2002. Relationships and generic circumscriptions in the *Psychotria* complex (Rubiaceae, Psychotrieae). Systematics and Geography of Plants 72, 167–202.

Andronova, N.N., 1977. On the structure of the ovule of Rubiaceae. Botanicheskii Zhurnal 62, 1461–1469.

Angenent, G.C., Colombo, L., 1996. Molecular control of the ovule development. Trends in Plant Science 1, 228–232.

Angenent, G.C., Stuurman, J., Snowden, K.C., Koes, R., 2005. Use of *Petunia* to unravel plant meristem functioning. Trends in Plant Science 10, 243–250.

Bouman, F., 1984. The ovule. In: Johri, B.M. (Ed.), Embryology of Angiosperms. Springer, Berlin, pp. 123–157.

Bouman, F., Calis, J.I.M., 1977. Integumentary shifting — a third way to unitegmy. Berichte der Deutschen botanischen Gesellschaft 90, 15–28.

Chiurugwi, T., Pouteau, S., Nicholls, D., Tooke, F., Ordidge, M., Battey, N., 2007. Floral meristem indeterminacy depends on flower position and is facilitated by a carpellate gynoecium development in *Impatiens balsamina*. New Phytologist 173, 79–90.

Colombo, L., Battaglia, R., Kater, M.M., 2008. Arabidopsis ovule development and its evolutionary conservation. Trends in Plant Science 13, 444–450.

De Toni, K.L.G., Mariath, J.E.A., 2004. Ovule development in *Borreria verticillata* (L.) G. Mey. (Rubiaceae — Rubioideae — Spermacoceae). Revista Brasileira de Botânica 27, 185–192.

De Toni, K.L.G., Mariath, J.E.A., 2008. Ovule ontogeny in Rubiaceae (Juss.): *Chomelia obtusa* (Cinchonoideae–Guettardeae) and *Ixora coccinea* (Ixoroideae–Ixoreae). Plant Systematics and Evolution 272, 39–48.

De Toni, K.L.G., Mariath, J.E.A., 2010. Ovule ontogeny of *Relbunium* species in the evolutionary context of Rubiaceae. Australian Journal of Botany 58, 70–79.

Doyle, J.A., Donoghue, M.J., 1986. Seed plant phylogeny and the origin of angiosperms: an experimental cladistic approach. The Botanical Review 52, 321–431.

Endress, P.K., 2011. Angiosperm ovule: diversity, development, evolution. Annals of Botany 107, 1465–1489.

Fagerlind, F., 1937. Embryologische, zytologische und bestäubungsexperimentelle Studien in der Familie Rubiaceae nebst Bemerkugen über einige Polyploiditätsprobleme. Acta Horti Bergiani 2, 196–470.

Gabriel, B.L., 1982. Biological Electron Microscopy. Van Nostrand Reinhold, New York.

Galati, G.G., 1991. Estúdios embriológicos em la tribu Spermacoceae (Rubiaceae). Parte I: Anatomia Floral. Megasporogénesis. Megagametogénesis. Boletín de La Sociedad Argentina de Botánica 27, 7–20.

Gerrits, P.O., Smid, L., 1983. A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. Journal of Microscopy 132, 81–85.

Heo, K., Van der Werff, H., Tobe, H., 1998. Embryology and relationships of Lauraceae (Laurales). Botanical Journal of the Linnean Society 126, 295–322.

Kimoto, Y., Utami, N., Tobe, H., 2006. Embryology of *Eusideroxylon* (Cryptocaryeae, Lauraceae) and character evolution in the family. Botanical Journal of the Linnean Society 150, 187–201.

Mariath, J.E.A., Cocucci, A.E., 1997. The ovules of *Relbunium hypocarpium* in the context of the Rubiaceae. Kurtziana 25, 141–150.

Martini, A.M.Z., Fiaschi, P., Amorim, A.M., Paixão, J.L., 2007. A hot-point within a hot-spot: a high diversity site in Brazil's Atlantic Forest. Biodiversity And Conservation 16, 3111–3128.

Nakagawa, H., Ferrario, S., Angenent, G.C., Kobayashi, A., Takatsuji, H., 2004. The *Petunia* ortholog of *Arabidopsis* SUPERMAN plays a distinct role in floral organ morphogenesis. The Plant Cell 16, 920–932.

O'Brien, T.P., Feder, N., McCully, M.E., 1965. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59, 368–373.

Oparka, K.J., Read, N.D., 1994. The use of fluorescent probes for studies of living plant cells. In: Harris, N., Oparka, K.J. (Eds.), Plant Cell Biology: A Practical Approach. Oxford University Press, London, pp. 27–50.

Razafimandimbison, S.G., Rydin, C., Bremer, B., 2008. Evolution and trends in the Psychotrieae alliance (Rubiacae) — a rarely reported evolutionary change of many-seeded carpels from one-seeded carpels. Molecular Phylogenetics and Evolution 48, 207–223.

Robbrecht, E., 1988. Tropical woody Rubiaceae. Characteristic features and progressions. Contributions to a new subfamilial classification. Opera Botanica Belgica 1, 1–271.

Robbrecht, E., Manen, J.F., 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (*n*DNA and *cp*DNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbc*L, *rps*16, *trn*L–*trn*F and *atp*B–*rbc*L data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. Systematics and Geography of Plants 76, 85–146.

Ronse Decraene, L.P.R., Smets, E.F., 2000. Floral development of *Galopina tomentosa* with a discussion of sympetaly and placentation in the Rubiaceae. Systematics and Geography of Plants 70, 155–170.

Sattler, R., Lacroix, C., 1988. Development and evolution of basal cauline placentation — *Basella rubra*. American Journal of Botany 75, 918–927.

Sattler, R., Perlin, L., 1982. Floral development of *Bougainvillea spectabilis* Wild., *Boerhaavia diffusa* L. and *Mirabilis jalapa* L. (Nyctaginaceae). Botanical Journal of the Linnean Society 84, 161–182.

Shamrov, I.I., 1998. Ovule classification in flowering plants — new approaches and concepts. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 120, 377–407.

Taylor, C., 2012. *Psychotria* in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (http://floradobrasil.jbrj.gov.br/2012/FB014153). Access in Apr. 10. 2012.

The Plant List, 2010. Version 1. Published on the Internet. (http://www.theplantlist.org/). Access in Apr. 10. 2012.

Tokuoka, T., Tobe, H., 1995. Embryology and systematics of Euphorbiaceae *sens. lat.* — a review and perspective. Journal of Plant Research 108, 97–106.

Tokuoka, T., Tobe, H., 2002. Ovules and seeds in Euphorbioideae (Euphorbiaceae): structure and systematic implications. Journal of Plant Research 115, 361–374.

Yamaki, S., Satoh, H., Nagato, Y., 2005. *Gypsy embryo* specifies ovule curvature by regulating ovule/integument development in rice. Planta 222, 408–417.

Zappi, D., 2003. Revision of *Rudgea* (Rubiaceae) in Southeastern and Southern Brazil. Kew Bulletin 58, 513–596.